Evidence for KCNQ1 K\(^+\) channel expression in rat zymogen granule membranes and involvement in cholecystokinin-induced pancreatic acinar secretion

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Lee W-K, Torchalski B, Roussa E, Thévenod F. Evidence for KCNQ1 K\(^+\) channel expression in rat zymogen granule membranes and involvement in cholecystokinin-induced pancreatic acinar secretion. Am J Physiol Cell Physiol 294: C879–C892, 2008. First published January 23, 2008; doi:10.1152/ajpcell.00490.2007.—Secretion of enzymes and fluid induced by Ca\(^{2+}\) in pancreatic acini is not completely understood and may involve activation of ion conductive pathways in zymogen granule (ZG) membranes. We hypothesized that a chromanol 293B-sensitive K\(^+\) conductance carried by a KCNQ1 protein is expressed in ZG membranes (ZGM). In suspensions of rat pancreatic ZG, ion flux was determined by ionophore-induced osmotic lysis of ZG suspended in isotonic salts. The KCNQ1 blocker 293B selectively blocked K\(^+\) permeability (IC\(_{50}\) of \(-10\) mM). After incorporation of ZGM into planar bilayer membranes, cation channels were detected in 645/150 mM potassium gluconate cis/trans solutions. Channels had linear current-voltage relationships, a reversal potential (E\(_{\text{rev}}\)) of \(-20.9 \pm 0.9\) mV, and a single-channel K\(^+\) conductance (g\(_{\text{K}}\)) of 265.8 \pm 44.0 pS (n = 39). Replacement of cis 500 mM K\(^+\) by 500 mM Na\(^+\) shifted E\(_{\text{rev}}\) to \(-2.4 \pm 3.6\) mV (n = 3), indicating K\(^+\) selectivity. Single-channel analysis identified several K\(^+\) channel groups with distinct channel behaviors. K\(^+\) channels with a g\(_{\text{K}}\) of 618.8 \pm 88.0 pS, E\(_{\text{rev}}\) of \(-22.9 \pm 2.2\) mV, and open probability (P\(_{\text{open}}\)) of 0.43 \pm 0.06 at 0 mV (n = 6) and channels with a g\(_{\text{K}}\) of 155.0 \pm 11.4 pS, E\(_{\text{rev}}\) of \(-18.3 \pm 1.8\) mV, and P\(_{\text{open}}\) of 0.80 \pm 0.03 at 0 mV (n = 3) were inhibited by 100 \mu M 293B or by the more selective inhibitor HMR-1556 but not by the maxi-Ca\(^{2+}\)-activated K\(^+\) channel (BK channel) inhibitor charybdotoxin (5 nM). KCNQ1 protein was demonstrated by immunoperoxidase labeling of pancreatic tissue, immunogold labeling of ZG, and immunoblotting of ZGM. 293B also inhibited cholecystokinin-induced amylase secretion of permeabilized acini (IC\(_{50}\) of \(-10\) mM). Thus KCNQ1 may account for ZG K\(^+\) conductance and contribute to pancreatic hormone-stimulated enzyme and fluid secretion.

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flufenamate (49). A K⁺ conductive pathway is blocked by ATP and glyburide (49, 50) and thus appears to be similar to ATP-sensitive K⁺ channels (23, 54). KCNJ11/Kᵢ₆.₂, the pore-forming subunit of ATP-sensitive K⁺ channels, is not expressed in rat pancreatic acini (46), although KCNJ8/Kᵢ₆.₁ has been recently detected in rat pancreatic ZG by immunoblotting (25). KCNQ1/K₈ɑ₁LQT1 may be another candidate for the ZG K⁺ channel. KCNQ1 (K₈ɑ₁LQT1; Kv7.1) is a very low-conductance (<1.5 pS), voltage-gated K⁺ channel distributed widely in epithelial and nonepithelial tissues (for review, see Refs. 4, 36). KCNQ1 was found to be mutated in the hereditary cardiac disease “long QT syndrome 1.” In the heart and inner ear, KCNQ1 coassembles with a β-subunit KCNE1 (Isk, minK) to form the outwardly rectifying and slowly activating, low-conductance K⁺ channel current (Iₖs). Iₖs are selectively blocked by chromanol 293B, which binds to KCNQ1 (45, 59).

In situ hybridization studies have shown that KCNQ1 is expressed in rodent pancreatic acini (12). Moreover, KCNQ1, which is the apical K⁺ channel required for active K⁺/H⁺ exchange and stimulated HCl secretion by gastric parietal cells, has also been detected in the intracellular tubulovesicles of parietal cells (18). In epithelial tissues, KCNQ1 K⁺ channels are activated by cAMP or Ca²⁺ and are selectively inhibited by chromanol 293B (4, 12, 18, 20, 29, 30, 45, 56).

In the present study, we tested the hypothesis that a 293B-sensitive K⁺ conductance carried by a KCNQ1 protein is expressed in rat pancreatic ZG membranes.

**EXPERIMENTAL PROCEDURES**

**Materials.** L-α-Phosphatidylcholine from soybean (asolecin) type II-S (P5638), protease inhibitor cocktail, Percoll, valinomycin, CCCP, collagenase (type III from Clostridium histolyticum, 790 U/mg), CCK octapeptide (CCK-OP), BSA (98–99%, essentially fatty-acid free), charybdotoxin, and goat anti-rabbit IgG coupled to 10-nm gold particles were purchased from Sigma (Deisenhofen, Germany). Trypsin inhibitor (from hen egg white) was obtained from Boehringer (Mannheim, Germany). Phedabase amylase kit was from Phadabase (Freiburg, Germany). Digitonin was from Fluka (Seelze, Germany). Glutaraldehyde, osmium tetroxide, and Araldit resin were purchased from Agar (Freiburg, Germany). Horseradish peroxidase (HRP)-conjugated donkey anti-rabbit IgG was from GE Healthcare (Little Chalfont, Buckinghamshire, UK), and goat anti-rabbit IgG coupled to HRP was obtained from Dako (Hamburg, Germany). Chromanol 293B and HMR-1556 were gifts from Sanofi-Aventis, Germany. All other reagents were of the highest analytical grade available.

**Isolation of ZG and purification of ZG membranes.** ZG from rat exocrine pancreas were isolated as described in detail elsewhere (6, 50, 52). Briefly, male Wistar rats (180–200 g, fasted overnight) were humanely killed by CO₂ anesthesia according to the ethical guidelines of the German state on animal welfare (approval no. 50.8735.1 Nr99/4).

Pancreatic tissue was immediately removed, homogenized by nitrogen pressure cavitation, and centrifuged on a self-forming Percoll gradient. ZG at the bottom of the centrifuge tube were washed in isotonic buffer containing 50 mM sodium succinate for removal of endogenous K⁺ conductive pathways before use (51). To obtain ZG membranes, a standard protocol was applied (50, 51) with slight modifications: ZG were resuspended on ice in a hypotonic lysis buffer (50 mM HEPES-Tris·HCl, 0.1 mM MgSO₄, pH 7.0) containing a protease inhibitor cocktail. The ZG were disrupted at 0 °C with the Branson Sonifier ultrasonic cell disruptor SS250A using a microtip probe (3 × 5-s pulses at 10 A), lysed by freezing at −80°C followed by rapid thawing, and centrifuged for 30 min at 196,000 g. The membrane pellet was either suspended in lysis buffer for immunoblotting or in 645 mM KCl for planar lipid bilayer experiments. Protein concentration was determined according to Bradford (5).

**Measurement of ion conductive pathways of rat pancreatic ZG.** Ion conductive pathways of rat pancreatic ZG were assayed according to previously reported protocols (49, 50). The assay for ion conductive pathways of pancreatic ZG relies on the measurement of osmotic lysis of ZG suspended in buffered isotonic salt solutions, which occurs after maximal permeabilization of the ZG membranes with electrogenic ionophores for counterions. Lysis is therefore limited by ion fluxes through endogenous conductive pathways. Granule lysis causes a decrease in absorbance of the suspension, which is measured at wavelength 540 nm. Measurements were carried out at 37°C in a Beckman DU-640 spectrophotometer equipped with a Peltier constant-temperature chamber and an automatic six-unit sampler. Data were captured and converted with DU-WinConnection Suite software.

For measurement of Cl⁻ conductive pathways, ZG were suspended in 150 mM KCl, 5 mM MgSO₄, and 50 mM HEPES (adjusted to pH 7.0 with Tris). Granule lysis was measured after addition of 5 μM valinomycin, an electrogenic K⁺ ionophore that maximally and selectively permeabilizes granule membranes for K⁺. The influx of salt and water causes ZG lysis and is limited under these conditions by the permeation of Cl⁻ through endogenous conductive pathways (10).

To determine K⁺ or Na⁺ conductive pathways (the latter being a measure of nonselective monovalent cation conductive pathways) (49), ZG were suspended in 150 mM potassium or sodium acetate containing 5 mM MgCl₂ and 1 mM EDTA and buffered with 50 mM imidazole (pH 7.0, adjusted with acetic acid). Because the intragranular pH is ~6.5 (50), an inside-to-outside directed H⁺ concentration gradient of ~0.5 pH units is generated across the granule membrane. After maximal permeabilization of the granule membrane to H⁺ by addition of the electrogenic protonophore CCCP (16 μM), the H⁺ concentration gradient is converted to an inside-negative H⁺ diffusion potential. This in turn drives K⁺ or Na⁺ influx through endogenous K⁺ and/or nonselective cation permeabilities (48, 49). Anion influx occurs through the uncharged molecule acetic acid, which permeates through the lipid membrane by nonionic diffusion and dissociates, thus continuously providing the intragranular space with protons for protonation of imidazole as well as for proton efflux from the acidic interior. Under these conditions, influx of monovalent cations through endogenous K⁺ and nonselective cation permeabilities is rate limiting for bulk salt influx into the intragranular space as well as for the resulting granule lysis.

Lysis rates were expressed as inverse half times of lysis, which were considered proportional to the lysis rate constants. Half time of granule lysis was estimated from the slope of decrease in absorbance with time between addition of ionophore and either experimental half time or the entire observation period if the half time was not reached. The slope of absorbance change with time was calculated by linear regression of the digitized data. At most, linear regression is an established approach for quantitative analysis of the kinetics of osmotic lysis in ZG, assuming that only one population of ZG is present. Stock solutions of chromanol 293B or HMR-1556 were dissolved in DMSO, which was added in the same concentration (0.1%) to the control cuvettes. Under these conditions, influx of monovalent cations through endogenous K⁺ and nonselective cation permeabilities is rate limiting for bulk salt influx into the intragranular space as well as for the resulting granule lysis.
decreasing end diameter. Acini were then purified by centrifugation in 4% BSA (wt/vol) and resuspended, and a final washing step was performed in the same buffer that is used for the secretion assay. Intact acini were incubated with 100% O$_2$ in a standard secretion buffer of the following composition (in mM): 135 KCl, 20 HEPES, 1.2 KH$_2$PO$_4$, 2 MgCl$_2$, 0.1 CaCl$_2$, 0.01% trypsin inhibitor (wt/vol), 0.2% BSA (wt/vol), and 10 glucose, pH titrated to 7.4 with KOH. To eliminate the contribution of plasma membrane monovalent cation transport pathways to amylase secretion, the plasma membrane was permeabilized by addition of 5 µg/ml digitonin to aliquots of acini in 2 ml of the standard secretion assay buffer for 10 min. Acini were subsequently incubated with a Ca$^{2+}$-dependent secretagogue CCK-OP (1 nM). After permeabilization, test substances or solvents (0.1% vol/vol) were preincubated for 5 min before stimulation with CCK-OP. Samples of 200-µl acinar suspension were taken shortly before and 30 min after addition of CCK-OP and centrifuged for 30 s at 14,000 rpm in an Eppendorf microfuge. The supernatant was removed, and amylase release from acini was determined using the Phadebas amylase test kit. To determine total amylase activity, portions of the residual samples were used. Acini were lysed in “diluent solution” containing 10 mM Na$_2$HPO$_4$, 10 mM NaH$_2$PO$_4$ buffer (pH 7.8), 0.1% SDS (wt/vol), and 0.1% BSA (wt/vol) for 1 h and further sonicated for 10 min. Amylase release was expressed as the percentage of total amylase activity present in the pancreatic acinar suspension. The percentage of amylase released during a period of 30 min in the absence of CCK-OP was subtracted for each value.

**1-o-Phosphatidylcholine purification.** 1-o-Phosphatidylcholine for the planar lipid bilayer technique was purified from asolectin by solvent extraction and purification procedures (19). Asolectin (2 g) from soybean was dissolved in 50 ml of chilled chloroform and mixed for 1 h at 4°C. The asolectin was subsequently precipitated with a fivefold excess of ice-cold acetone and mixed for a further 1 h at 4°C. The purified lipid was aliquoted and collected by centrifugation at 1,100 g for 15 min at 4°C. Excess acetone was decanted, and 1-o-phosphatidylcholine was stored at −80°C under N$_2$. Upon reconstitution, purified 1-o-phosphatidylcholine was weighed, dried under N$_2$ to remove any remnants of acetone, weighed again, and dissolved to a final concentration of 25 mg/ml in n-decane.

**Planar lipid bilayer technique.** The planar lipid bilayer was set up as described elsewhere (19, 34). A planar lipid membrane was formed by spreading phospholipid dispersions on a 250-µm diameter hole, which separates two chambers (cis and trans) with 1 ml of internal volume) (Hugo Sachs Elektronik, Harwood Apparatus, Hugstetten, Germany). The cis compartment, defined as the compartment to which ZG membrane vesicles were added, contained 645 mM KCl or potassium gluconate and 10 mM HEPES (pH 7.2 adjusted with Tris). The trans compartment, connected to the virtual ground of the amplifier, contained 150 mM KCl or potassium gluconate and 10 mM HEPES (pH 7.2). For ion selectivity experiments, 500 mM potassium gluconate was replaced by 500 mM sodium gluconate in the cis chamber only. Nonpolarizing electrodes (Ag/AgCl pellets) immersed in 3 M KCl were used to connect each side of the bilayer to the headstage of a BLM-120 bilayer clamp amplifier (BioLogic, Claix, Union City, CA). To determine single-channel conductance, all-points histograms were generated. The peak amplitude data were grouped into user-defined bins (amplitude steps) to elicit a histogram. Each histogram peak was fitted further by Gaussian distribution fit to determine the mean amplitude of each channel level. To account for the concentration gradient of permeable ions between the cis and trans chambers, channel conductance was determined with the modified current-voltage law (21): \( \mathcal{I}_c = \frac{g_c}{\eta_c} (E - E_K) \), where \( \mathcal{I}_c \) = K$^+$ current, \( g_c \) = K$^+$ conductance, \( E \) = holding potential, and \( E_K \) = reversal potential of K$^+$ at 20°C.

For the majority of experiments, more than one channel was reconstituted into the bilayer. With the assumption that the different levels were multiple openings of the same channel type, the open probability (\( P_{\text{open}} \)) was computed as sum of total open time \( \times \) level/total time/number of channels, where number of channels is the number of levels. This is referred to as “simple \( P_{\text{open}} \)” in Clampfit (pCLAMP, version 9.2, Axon Instruments; Union City, CA). To determine single-channel conductance, all-points histograms were generated. The peak amplitude data were grouped into user-defined bins (amplitude steps) to elicit a histogram. Each histogram peak was fitted further by Gaussian distribution fit to determine the mean amplitude of each channel level. To account for the concentration gradient of permeable ions between the cis and trans chambers, channel conductance was determined with the modified current-voltage law (21): \( \mathcal{I}_c = \frac{g_c}{\eta_c} (E - E_K) \), where \( \mathcal{I}_c \) = K$^+$ current, \( g_c \) = K$^+$ conductance, \( E \) = holding potential, and \( E_K \) = reversal potential of K$^+$ at 20°C.

Data acquisition. Unitary current, dwell time and gating measurements, statistical analysis, and data processing were performed by using commercially available software packages (pCLAMP, version 9.2; and Sigma Plot 8.0, SPSS, Chicago, IL).

**Antibodies.** The KCNQ1 antibody (BLE 2-1) was obtained by immunizing rabbits with a peptide (CPADLGPPRYSLDPVRSY) of the cytosolic NH$_2$ terminus (residues 67–94 of rat KCNQ1; accession no. CH473953.1) (18, 20). An additional antiserum against KCNQ1 was raised in rabbits against a peptide that represents a part of the COOH terminus of human KCNQ1 (CLTVPTQPGDEGS; residues 658–669; accession no. AY114213.1) (11, 13). A commercial antibody (AB5587-50UL) was raised against a peptide corresponding to residues 661–676 of human KCNQ1 (accession no. P51787), which does not react with other QKT proteins. All KCNQ1...
antibodies were affinity purified. Goat anti-rabbit IgG coupled to HRP for immunohistochemistry was used as secondary antibody, goat anti-rabbit IgG coupled to 10-nm gold particles for immunogold labeling, and a HRP-conjugated donkey-anti-rabbit IgG for immunoblotting.

**Immunohistochemistry.** Rat pancreas was perfusion fixed with 4% paraformaldehyde (wt/vol), cut in small pieces, cryo-protected, and frozen in liquid N\textsubscript{2}. Immunohistochemistry was performed on 5-µm cryosections. Slides were treated with 1% SDS (wt/vol) for 5 min, blocked with 1% BSA (wt/vol)-PBS for 15 min, and incubated with rabbit polyclonal antibodies against human KCNQ1 at 1:20 dilution overnight at 4°C. After incubation with goat anti-rabbit IgG coupled to HRP (1:50), peroxidase reaction was visualized as described elsewhere (38).

**Preembedding immunogold electron microscopy.** Electron microscopy of isolated rat pancreatic ZG was performed as described earlier (52) with minor modifications. ZG were fixed in 2.5% glutaraldehyde in PBS for 1 h and adsorbed onto poly-L-lysine-coated glass slides. Grains were washed with PBS, treated sequentially with PBS containing 0.5% BSA, 0.5% gelatin, and 0.5% TWEEN 20, followed by PBS containing 0.05% BSA, 0.05% gelatin, and 0.05% TWEEN 20, and then incubated with BLE 2-1 (1:30 dilution) overnight at 4°C. Samples were incubated with goat anti-rabbit IgG coupled to 10-nm gold particles (1:10 dilution) for 1 h at room temperature, fixed with 1% glutaraldehyde in PBS for 30 min, and washed with water for 45 min. Samples were postfixed with 1% OsO\textsubscript{4} in 0.1 M sodium cacodylate, treated with aqueous 2% uranyl acetate, dehydrated in a graded series of ethanol, and embedded in Araldit resin. Thin sections were counterstained with 2% uranyl acetate and lead citrate and viewed with a Philips EM-10 electron microscope.

**Immunoblotting.** Twenty-five micrograms of protein of rat pancreas tissue homogenate or ZG membranes were mixed with 3% Laemmli buffer, incubated for 5 min at 95°C, and sonicated on ice. Membrane proteins were separated by SDS-PAGE on 9% acrylamide Laemmli minigels and transferred onto polyvinylidene difluoride membranes. After blocking with Tris-buffered saline containing 0.1% Tween 20 and 3% non-fat dry milk for 8 h, the membranes were incubated at 4°C with BLE 2-1 (1:1,000) overnight, followed by HRP-conjugated donkey anti-rabbit IgG (1:10,000) for 1 h. The blots were developed using unpaired Student’s t-test was carried out with Sigma Plot 8.0 (SPSS). For more than two groups, one-way ANOVA was used, assuming equality of variance with Levene’s test and Tukey’s post hoc test for pairwise comparison with SPSS 12.0. Results with \( P \leq 0.05 \) were considered to be statistically significant.

**RESULTS**

**Differential inhibition of ZG ion conductive pathways by chromanol 293B.** The membrane of ZG is equipped with at least two cation and two anion conductive pathways, which contribute to K\textsuperscript{+} and Cl\textsuperscript{−} fluxes into the granule matrix and promote the release of digestive enzymes (for review, see Ref. 48). The osmotic lysis assay of isolated ZG represents a simple and robust technique to measure macroscopic flux of ions across the ZG membrane. It also permits screening of drugs with high throughput but has the disadvantage of low sensitivity and specificity when it comes to identifying ion channels (58). As a first approach to determine whether KCNQ1-type K\textsuperscript{+} channels contribute to ZG K\textsuperscript{+}-conductance pathways, we investigated the effect of the chromanol 293B on ZG K\textsuperscript{+} conductance. In the absence of the electrogenic protonophore CCCP (16 µM), the absorbance of ZG suspended in 150 mM potassium acetate and buffered with 50 mM imidazole to pH 7.0 remains stable throughout the recording period when K\textsuperscript{+} or other monovalent cations are used as major osmolytes (49, 50) (Fig. 1, A and B; data not shown). Addition of CCCP decreases the absorbance of the ZG suspension as a result of increased osmotic lysis of ZG (Fig. 1A). This is due to CCCP-induced influx of K\textsuperscript{+} and acetic acid, which is driven by the inside-negative H\textsuperscript{+} diffusion potential (pH of ZG matrix < pH of the buffer) (see EXPERIMENTAL PROCEDURES and Ref. 50). As shown in Fig. 1, A and D, 293B inhibited ZG lysis in K\textsuperscript{+} buffer in a concentration-dependent manner from 0.1 to 100 µM 293B. Inhibition of ZG lysis was half-maximal at ∼10 µM (Fig. 1D) and blocked 70–80% of ZG lysis at 100 µM 293B, but ∼20–30% of K\textsuperscript{+} conductive pathway was not affected by 293B (Fig. 1A). Moreover, HMR-1556 (0.1–100 µM), another specific inhibitor of KCNQ1-type K\textsuperscript{+} channels (15, 17, 32), also inhibited the ZG K\textsuperscript{+} conductive pathway with a similar potency (data not shown). When K\textsuperscript{+} is used as the major osmolyte, both K\textsuperscript{+}-selective and a nonselective monovalent cation permeability pathway contribute to overall ZG lysis (49). Therefore, we investigated the effect of 293B on the nonselective cation pathway by replacing K\textsuperscript{+} with Na\textsuperscript{+}, which equally permeates the nonselective cation conductive pathway (49). As shown in Fig. 1, B and D, 293B even at 100 µM had a very small inhibitory effect (∼20% inhibition) on ZG lysis, indicating that the 293B-insensitive ZG lysis in K\textsuperscript{+} buffer can be accounted for by the nonselective cation permeability pathway.

Finally, we investigated the effect of 293B on the ZG Cl\textsuperscript{−} permeability pathway (Fig. 1C). In the absence of the electrogenic potassium ionophore valinomycin (5 µM), the absorbance of the ZG suspension remains stable throughout the recording period when 150 mM KCl is used as major osmolyte (49). Addition of valinomycin decreases the absorbance of the ZG suspension as the result of increased osmotic lysis of ZG due to valinomycin-mediated influx of K\textsuperscript{+} and flux of anions through endogenous electrogenic ion pathways. However, 293B had no effect on ZG lysis in Cl\textsuperscript{−} buffer (Fig. 1, C and D). Hence, the data demonstrate that, in rat pancreatic ZG, 100 nM 293B already inhibits ZG lysis in K\textsuperscript{+} buffer and complete inhibition is observed at 10 µM 293B, whereas the nonselective monovalent cation and the Cl\textsuperscript{−} conductive pathways are not affected by up to 100 µM of the drug.

**Biophysical characterization of ZG membrane K\textsuperscript{+} channels incorporated into planar bilayers and pharmacological block by 293B and HMR-1556.** From 11 individual preparations of rat ZG membranes, 472 experiments were performed. After incorporation of ZG membranes, channel events were frequently observed (i.e., in ∼20–25% of all experiments). However, in only 48 experiments were full-range current-voltage relationships available, allowing detailed biophysical and pharmacological analyses. Only these 48 experiments were included in the study. Of these, five single channels were observed with KCl buffer in both chambers. The reversal potential (\( E_{rev} \)) of these five single channels was \( -15.3 \pm 4.5 \) mV (compared with a theoretical Cl\textsuperscript{−} potential of +36.8 mV or a theoretical \( E_k \) of 36.8 mV based on the Nernst equation with 645 mM KCl cis and 150 mM KCl trans). Obviously, the currents were more K\textsuperscript{+} than Cl\textsuperscript{−} selective. However, we could not exclude the superpositioning of currents from simultaneously incorporated Cl\textsuperscript{−} and K\textsuperscript{+} channels, which would
affect $E_{\text{rev}}$ toward nonselectivity. Clearly, in some of the KCl experiments, the estimated $E_{\text{rev}}$ was about $-30.0$ mV, showing near “ideal” K+ selectivity. Apart from K+ channels, anion-selective channels were also occasionally recorded. In the two experiments where current-voltage relationships were obtained, the respective $E_{\text{rev}}$ values were $+33.6$ and $+34.5$ mV, indicating near perfect Cl− selectivity (data not shown). However, these channels were very rarely observed and were not investigated further. To prevent simultaneous recordings of Cl− and K+ currents, we replaced Cl− with gluconate ion to focus on the more frequently observed K+ channels (in addition, we sometimes observed that the bilayer itself displayed a nonspecific background permeability, which could have also affected $E_{\text{rev}}$ in some experiments; data not shown).

Under potassium gluconate buffer conditions, the $E_{\text{rev}}$ of single channels from all experiments recorded was $-20.9 \pm 0.9$ mV ($n = 39$) (theoretical $E_{K} = -36.8$ mV). The average single-channel conductance of these K+ selective channels was $265.8 \pm 44.0$ pS when measured in the range of $-30$ to $+30$ mV with 645 mM/150 mM potassium gluconate cis/trans. The mean single-channel $P_{\text{open}}$ was estimated at 0.49 $\pm$ 0.05 (Table 1). To ensure that the K+ currents were not due to nonselective cation channels [a flufenamate-sensitive nonselective monovalent cation permeability has been described in ZG with the osmotic lysis assay, which is equally selective to K+ and Na+ (48, 49); Fig. 1], we replaced 500 mM potassium gluconate in the cis chamber with 500 mM sodium gluconate. This gradient of 145/150 mM potassium gluconate cis/trans should shift $E_{\text{rev}}$ of K+ selective channels toward more positive values ($E_{K} = +0.9$ mV), whereas $E_{\text{rev}}$ of a nonselective monovalent cation channel would remain unaffected by this change of solution. As shown in Table 1, $E_{\text{rev}}$ under 145/150 mM potassium gluconate cis/trans was $-2.4 \pm 3.6$ mV ($n = 3$), and the single-channel conductance decreased to 105.3 $\pm$ 23.8 pS. This indicates that the ZG membrane channels observed are K+ selective.

These large K+ channels were not typical for KCNQ1 K+ channels (4, 36), although the large conductances observed could partly be accounted for by the artificially high K+ concentration used in the cis chamber (645 mM) and/or by possible interaction of putative KCNQ1 K+ channels with channel subunits that could modify the properties of the channel (see DISCUSSION). However, in view of the large conductances of the K+ channels, we also investigated whether they

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Fig. 1. Effect of 293B on K+-, Na+, and Cl− conductive pathways of rat pancreatic zymogen granules (ZG) suspended in buffered isosmotic salt solution. A: protonophore-induced osmotic lysis of ZG suspended in 150 mM potassium acetate buffered with 50 mM imidazole (pH 7.0) and inhibition by different concentrations of 293B. ZG were suspended in the buffer (1st arrow) with or without (0.1% DMSO) the indicated concentrations of 293B. The protonophore CCCP (16 μM) was added (2nd arrow) to induce K+ conductance (for details of the lysis assay, see EXPERIMENTAL PROCEDURES). B: to assay nonselective monovalent cation conductive pathways, ZG were suspended in 150 mM sodium acetate buffered with 50 mM imidazole (pH 7.0). Otherwise, experimental conditions were identical to those in A. C: to assay Cl− permeability, ZG were resuspended in 150 mM KCl buffered with 50 mM HEPES (pH 7.0), and osmotic lysis was induced by adding 5 μM of the electrogenic potassium ionophore valinomycin (2nd arrow). Dotted traces represent control experiments in the absence of ionophore. All curves are typical for 3–5 experiments. D: dose-response curve for block of ion permeabilities by 293B in rat pancreatic ZG. Experiments were conducted in the absence or presence of different concentrations of the KCNQ1 blocker 293B. Control rates (100%) of granular lysis after addition of ionophores were measured in the absence of 293B but with 0.1% DMSO in the buffer and were estimated to be 10.6 $\pm$ 3.5 h$^{-1}$ for K+ permeability, 4.8 $\pm$ 2.9 h$^{-1}$ for Na+ permeability, and 3.3 $\pm$ 0.7 h$^{-1}$ for Cl− permeability. Results are means $\pm$ SE of 3–5 different experiments.
might represent BK channels. Charybdotoxin, a scorpion venom toxin that blocks BK channels with high affinity ($K_a$ of $\sim$2 nM) (16, 35), was applied at a concentration of 5 nM to both cis and trans chambers because the orientation of the $K^+$ channel in the bilayer membrane was unknown. However, charybdotoxin had no effect on single-channel $P_{\text{open}}$ (Table 1) and current amplitude (data not shown) in five different experiments, which argues against BK channels being responsible for the ZG membrane $K^+$ currents. Although BK channels are activated by $Ca^{2+}$, this could not be tested in this experimental setup because $Ca^{2+}$ is also fusogenic and enhances ZG membrane vesicle fusion with the planar bilayer (data not shown). A closer analysis of the data showed that $K^+$ channel currents observed in potassium gluconate could be split into several groups based on their channel properties and frequency of occurrence. The biophysical properties of these groups of $K^+$ channels are summarized in Table 2. Other channels, however, could not be attributed to any of the groups described below.

The first group of $K^+$ channels (named $K^+$ group I) had a large current and peak amplitude ($25–30$ pA at 0 mV), and the single-channel conductance was $651.8 \pm 88.0$ pS ($n = 6$) (Fig. 2A and Table 2). The recorded currents mostly showed multiple levels of channel events, and up to four open levels were observed, as exemplified in the experiment shown in Fig. 2A and the respective all-points histogram at 0 mV (Fig. 2B). To determine whether the multiple levels of channel events observed represented opening of different channels or were due to opening of several identical channels, open-channel histograms of the data obtained at 0 mV were performed as described in EXPERIMENTAL PROCEDURES. The majority of events were caused by one set of channels with single peak amplitudes of $\sim 20–40$ pA at 0 mV; occasionally, another set of smaller channels with a peak amplitude of $5–7$ pA was detected (data not shown). Assuming that the different levels were multiple openings of the same channel type, the single-channel $P_{\text{open}}$ was calculated to be $0.43 \pm 0.06$ at 0 mV ($n = 6$) (Table 2). The channels had relatively long dwell times of $\sim 60$ ms at 0 mV. Open and closed transition events of these channels appeared to be almost equivalent in time lapse, as depicted by the near equal $\tau$ results for both opening and closing phases (Table 2). Interestingly, additional current levels at 0 mV were more likely to be observed after hyperpolarization rather than after depolarizing steps, whereas the probability of the first channel level to be active was independent of the preceding voltage step (data not shown). Figure 2C summarizes the current-voltage relationship of the channels obtained in six experiments. It is interesting to note that these $K^+$ channels ($E_{\text{rev}} = -22.9 \pm 2.2$ mV) had a linear current-voltage relationship, which differs from the $I_K$ associated with KCNQ1/ KCNE1 $K^+$ channels (3, 39). In five or six experiments, the specific KCNQ1 blockers 293B and HMR-1556 (15, 17, 32) inhibited the $K^+$ channel current observed. A representative experiment is depicted in Fig. 2D, where channel events were recorded at 0 mV and four channel level openings can be seen. The inhibitor HMR-1556 (100 μM) was applied with constant stirring to both cis and trans chambers because the orientation of the $K^+$ channel in the bilayer membrane was unknown. As seen in Fig. 2D, HMR-1556 effectively blocked the $K^+$ channels. Immediately after inhibitor application, the multiple channel opening levels gradually decreased to reach a maximal inhibitory effect after 1.5 min where only one channel level opening was present (Fig. 2D, bottom trace). Accordingly, single-channel $P_{\text{open}}$ was significantly reduced by $\sim 70\%$ with 100 μM HMR-1556 (Fig. 2E). Similar observations were made with 293B (data not shown and Table 1).

Table 2. Gating properties of $K^+$ groups I–III channels in zymogen granules

<table>
<thead>
<tr>
<th>Group</th>
<th>$E_{\text{rev}}, \text{mV}$</th>
<th>Conductance, pS</th>
<th>Time Constant, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Open</td>
<td>Closed</td>
</tr>
<tr>
<td>Group I ($n = 6$)</td>
<td>$-22.9 \pm 2.2$</td>
<td>$651.8 \pm 88.0$</td>
<td>$4.39 \pm 0.8$</td>
</tr>
<tr>
<td>Group II ($n = 3$)</td>
<td>$-23.2 \pm 1.1$</td>
<td>$57.6 \pm 28.9$</td>
<td>$2.69 \pm 0.7$</td>
</tr>
<tr>
<td>Group III ($n = 3$)</td>
<td>$-18.3 \pm 1.8$</td>
<td>$155.0 \pm 11.4$</td>
<td>$1.69 \pm 0.2$</td>
</tr>
</tbody>
</table>

Values are means ± SE. The properties of each channel group observed in zymogen granules (see Figs. 2–4) were analyzed and determined as described in the legend to Table 1 and in EXPERIMENTAL PROCEDURES. All channels were analyzed from recordings at 0 mV, apart from group II where single-channel activity could only be accurately analyzed at more positive voltages and data were analyzed from recordings at +40 mV.

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Another group of $K^+$ channels in ZG membranes with multiple levels was named $K^+$ group II (Fig. 3A). These $K^+$ currents consisted of only one population of $K^+$ channels based on analysis using an all-points histogram (Fig. 3C). Moreover, multiple channel level openings were only observed at higher positive voltages from -40 to +60 mV. The current-voltage relationships and $E_{rev}$ ($-23.2 \pm 1.1$ mV; $n = 3$) were similar to results for $K^+$ group I (Fig. 3B). However, the $K^+$ channels had a comparatively small current at 0 mV (2–3 pA), a single-channel conductance of 57.6 ± 28.9 pS, and a $P_{open}$ of 0.38 ± 0.09 at +40 mV ($n = 3$) (Table 2). Channel openings had a dwell time of ~30 ms, and, similar to that shown in $K^+$ group I, the opening and closing results were almost identical to each other (Table 2).

Finally, a further set of $K^+$ channels in ZG membranes was identified (named $K^+$ group III) (Fig. 4, A and B). These

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Fig. 2. Single-channel activity and biophysical and pharmacological properties of $K^+$ channels ($K^+$ group I) in ZG membranes using planar lipid bilayer. ZG membrane vesicles were fused with planar lipid bilayers, and currents were recorded at different voltage steps in 645/150 mM potassium gluconate cis/trans. Channel activity was recorded for up to 1 min. Closed state (C) and different open-channel levels (O1–O4) are indicated. Scales are applicable to all traces for each channel type. A: $K^+$ group I channels are large $K^+$-conducting channels with frequent openings and closings and show multiple levels of opening (up to 4). Typical traces of 6 individual experiments are shown. B: respective all-points histogram at 0 mV. C: current-voltage relationship of $K^+$ group I channels was fitted by linear regression. Results are means ± SE of 6 experiments. Reversal potential ($E_{rev}$; $-22.9 \pm 2.2$ mV) was determined from each fitted plot. D: to test the effect of HMR-1556 (100 μM), currents were recorded at 0 mV. Channel activity was recorded for 4 min after addition of the inhibitor. E: single-channel open probability ($P_{open}$) was derived from $NP_{open}/N$ (where $N$ is no. of open channels), assuming that the different levels represent multiple openings of the same channel type based on the data from the single-channel search analysis in Clampfit. Results are means ± SE of 6 experiments with HMR-1556. Statistical analysis using Student’s paired $t$-test compared single $P_{open}$ before and after inhibitor application.
channels were distinct from the previous channels in $K^+$ groups I and II seen so far. They had a linear current-voltage relationship with an $E_{\text{rev}}$ of $-18.3 \pm 1.8$ mV ($n = 3$) and an average conductance of $155.0 \pm 11.4$ pS at 0 mV ($n = 3$) (Fig. 4C and Table 2). At 0 mV, the channel had a high $P_{\text{open}}$ (0.80 $\pm$ 0.03), which increased further at more positive voltages (>0.9) and showed a characteristic flickering pattern of opening and closing. The all-points histogram revealed numerous sublevel conductances (Fig. 4B). Open channels had a short dwell time of $\sim$8 ms, which further supports the rapid open-close activities of these channels. In contrast to $K^+$ groups I and II, the closing time constants of $K^+$ group III channels were slower than opening transitions according to the $\tau$ values (Table 2). When 100 $\mu$M 293B was applied to this $K^+$ channel from both cis and trans sides at a holding potential of $-50$ mV, the channel was completely inhibited immediately, but a 293B-resistant $K^+$ current was revealed, which, however, showed different gating properties (Fig. 4D, inset, Table 1, and data not shown). This 293B-resistant current had a peak amplitude of $\sim$1.0 pA at $-50$ mV, and $P_{\text{open}}$ was decreased to $-0.07$ (Fig. 4D). The inhibitor 293B had a potent effect on blocking $K^+$ channel $P_{\text{open}}$, significantly reducing it by $>80\%$ (Fig. 4E). A similar behavior was also observed at other voltages (data not shown).

Localization of KCNQ1 in ZG of rat pancreatic acinar cells. Figure 5A illustrates the labeling pattern of KCNQ1 in rat pancreas using the antibody raised against amino acids 661–676 of human KCNQ1 (1:20 dilution) and immunoperoxidase light microscopy. Acinar cells showed diffuse labeling of moderate intensity restricted to the apical cell pole of the cells, at the area occupied by the ZG, presumably representing immunolabeling of ZG (arrows in Fig. 5A), as well as weak labeling of the basolateral plasma membrane in some cells. In the absence of primary antibody, no staining was observed (Fig. 5A, −Ab). Similar results were obtained with an antibody against amino acids 658–669 of human KCNQ1 (11) (data not shown). Localization of KCNQ1 in rat ZG was also investigated at the ultrastructural level to confirm and extend the immunostaining results. Rat pancreatic ZG were also isolated and processed for silver-enhanced preembedding immunogold labeling and electron microscopy with the BLE 2-1 antibody (dilution 1:30). Figure 5B clearly demonstrates that the gold particles were exclusively located at the membrane surface of the granules, which provides further evidence for the presence of KCNQ1 in ZG membranes and confirms the results obtained by immunoblotting. Immunogold labeling was absent in controls, in which the primary antibody was omitted (Fig. 5B, −Ab).

Detection of KCNQ1 in ZG membranes of rat pancreas by immunoblotting. Cloning of rat KCNQ1 has revealed a gene product that codes for a 669-amino acid protein (30), which is expressed in rat pancreatic acini (56). Human KCNQ1 has 676 amino acids and is also detected by the BLE 2-1 rabbit polyclonal antibody in immunoblots, where it migrates at $\sim$75 kDa (18). The immunoblotting experiments on ZG membranes with BLE 2-1 (1:100) are shown in Fig. 5C. The anti-KCNQ1 antibody recognized a band of $\sim$80 kDa in rat pancreas homogenate that was enriched in ZG membranes. The antibody detected an additional band of a size slightly higher than 50 kDa in homogenate and ZG membranes that was also present in controls without primary antibody (Fig. 5C, −Ab).
Inhibition of CCK-OP-induced amylase secretion by 293B in permeabilized pancreatic acini. To study the possible role of KCNQ1 in secretagogue activation of pancreatic enzyme secretion, we tested various concentrations of the inhibitor of KCNQ1, chromanol 293B, on basal and CCK-OP-induced amylase secretion of isolated pancreatic acini. Acini were permeabilized with 50 μg/ml digitonin before hormonal stimulation to exclude a possible contribution of KCNQ1/KCNE1 K+ channels expressed in the plasma membrane of rat pancreatic acinar cells (56). Amylase released into the medium within 30 min under basal conditions or after addition of a maximally stimulatory concentration of 1 nM CCK-OP was calculated as a percentage of the total amount of amylase in the cell suspension (1). Under basal conditions, amylase release was similar in buffers without and with 293B. In DMSO controls, 3.1 ± 0.4% of total amylase was released (n = 5), whereas in the presence of the maximal tested concentration of 100 μM 293B, the release amounted to 2.9 ± 0.6% of total amylase (n = 4) (data not shown). CCK-OP (1 nM) stimulated amylase release in control acini (3.7 ± 0.7% of total amylase above basal; n = 5). Quite unexpectedly, 293B (1–100 μM) did not affect CCK-OP-stimulated amylase release (e.g., 3.4 ± 0.4% of total amylase above basal at 100 μM 293B; n = 7) (see Fig. 6). However, both the K+ conductive and the nonselective monovalent cation conductive pathway of ZG are permeable to K+ (49). Therefore, even if 293B blocked the K+–selective channel, K+ could still enter the granule matrix via a nonselective channel and thereby enhance CCK-OP-evoked enzyme secretion. Flufenamic acid has been shown to specifically inhibit the nonselective monovalent cation permeabilities of ZG but has no effect on ZG K+ and Cl– conductive pathways (Ref. 49 and data not shown). Hence, we repeated the experiments in the presence of 100 μM flufenamic acid. Basal amylase release was not affected by 100 μM flufenamate (3.3 ± 0.8% of total amylase, n = 3; data not shown). With 100 μM flufenamate, CCK-OP (1 nM)-stimulated amylase secretion was slightly increased (4.7 ± 0.7% of total amylase above basal; n = 5) but did not reach significance. In contrast, when 100 μM flue-
cific antibodies, which are directed against different cytosolic NH2- and COOH-terminal epitopes of rodent (18) and human KCNQ1 (11, 13). Moreover, several lines of functional evidence indicate that 293B- and HMR-1556-sensitive K+ channels associated with KCNQ1 may underlie ZG K+selective monovalent K+ conductive pathways of ZG and contribute to secretagogue-induced enzyme secretion from pancreatic acini.

**293B inhibitor specificity and proposed site of action.** The interpretation of the data in Fig. 1 is critically dependent on the relative permeability for K+ over Na+ of the K+ channel studied, the relative permeability for K+ over Na+ of the nonselective cation channel, and the efficacy of 293B to block the K+ channel as opposed to the nonselective cation channel. In fact, 293B was found to affect lysis rates in K+ buffer at much lower concentrations than lysis in Na+ buffer (Fig. 1), suggesting that the bulk of the lysis effect is due to influx via the K+ channel. It has been suggested that 293B might have the properties of a protonophore. The osmotic lysis assay for cation conductive pathways depends on an H+ gradient. Hence, it could be argued that the inhibitory effect of 293B on K+ permeability might be caused by dissipation of the H+ gradient across the ZG membrane rather than by specific inhibition of a KCNQ1 channel. However, ZG lysis in Na+ buffer was not affected by 293B (Fig. 1). Moreover, Grahammer et al. (18) have directly shown by BCECF fluorescence measurements of intact cells that 293B does not act as a protonophore. In addition, fusion of ZG membranes with planar bilayers resulted in the appearance of K+-selective channels (Figs. 2–4) that were inhibited by 293B or by HMR-1556, which specifically inhibits KCNQ1 channels (15, 17, 32). Finally, CCK-OP-induced secretion of amylase by permeabilized pancreatic acini was efficiently blocked by 293B with an IC50 of ~10 μM, when the inhibitor of the nonselective

![Image](http://ajpcell.physiology.org/)

**DISCUSSION**

The present study indicates that KCNQ1 (KvLQT1; Kv7.1) is expressed in ZG membranes of the rat exocrine pancreas (Fig. 5), as demonstrated by immunoblotting of ZG membranes, immunostaining of pancreatic acini, and immunogold electron microscopy labeling of isolated ZG with several spe-

![Image](http://ajpcell.physiology.org/)

**Fig. 5.** Expression of KCNQ1 in pancreatic ZG membranes. A: immunolocalization of KCNQ1 in ZG (arrows) of rat pancreatic acinar cells using an antibody raised against human KCNQ1 (1:20) and immunoperoxidase light microscopy (+Ab). No labeling was detectable when tissue sections were incubated with the secondary antibody alone (−Ab). B: preembedding immunogold electron microscopy of isolated rat pancreatic ZG with KCNQ1 antibody BLE 2-1 (1:30) directed against rodent KCNQ1 (+Ab). Immunogold staining was absent in controls, i.e., in the absence of primary antibody (−Ab). Bar = 0.2 μm. C: immunoblotting of rat pancreatic homogenate (Ho) and ZG membranes (ZGM). Membrane protein (25 μg) was probed with the polyclonal antibody BLE 2-1 raised against KCNQ1 (1:1,000). One representative immunoblot of 3 similar ones is shown. In the absence of primary antibody (−Ab), a nonspecific protein band of >50 kDa molecular mass (MM) was also detected in ZGM.

![Image](http://ajpcell.physiology.org/)

**Fig. 6.** Effect of 293B on CCK octapeptide (CCK-OP)-induced amylase secretion of permeabilized rat pancreatic acini. Acini were permeabilized with 5 μg/ml digitonin, incubated in buffer with 135 mM KCl, and preincubated for 5 min with inhibitors or 0.1% solvent before addition of the secretagogue. The effect of different concentrations of 293B on CCK-OP-induced secretion of amylase was subtracted from the respective values with CCK-OP. Values are means ± SE of 3–7 experiments. *Significant differences (P < 0.01) between experiments without or with 293B using one-way ANOVA.
cation conductance flufenamate was also present in the medium (Fig. 6), indicating that ZG cation conductances are required for enzyme secretion to occur.

**Characteristics of ZG K⁺ channels.** Kelly et al. (25) were able to patch single pancreatic ZG and detected ion conductances with KCl in the bath and patch pipette that were partially inhibited by low concentrations (20–40 μM) of the Cl⁻ channel blocker DIDS and K⁺ channel blockers quinine and glyburide. Single-channel currents were also observed, which were nonselective but inhibited by 20 μM DIDS. Channels had an intermediate conductance of ~40 pS and no voltage dependence. Immunoblots revealed expression of the Cl⁻ channel proteins CIC-2 and CIC-3 and of the K⁺ channel protein KCNJ8/Kv6.1. This study was hampered by the use of relatively unspecific inhibitors but still represents the first direct electrophysiological evidence for ion channels in ZG membranes. In the present study, we provide evidence for the presence of a KCNQ1 K⁺ channel protein in ZG membranes (Fig. 5) in conjunction with K⁺-selective single channels incorporated into planar bilayers, which are blocked by the specific inhibitors of KCNQ1, 293B and HMR-1556 (Figs. 2–4).

Although the solution replacement experiments (Table 1) indicated that the monovalent cation channels observed were indeed K⁺ selective, the large conductances observed are unusual for KCNQ1 K⁺ channels (4, 36). This anomaly may well be accounted for by possible interaction of KCNQ1 with specific, yet unknown, KCNQ1 channel subunits, which could modify the properties of the channel (37) (see also below). However, this remains unproven. Another important fact that could account for the large K⁺ conductance is that the K⁺ concentration used in the study in the cis chamber (645 mM) is much higher than those used in published patch-clamp studies. In those studies (37, 43), 100–140 mM K⁺ was used and conductances between 7 and 16 pS were measured. Moreover, external (trans) K⁺ concentration may also affect KCNQ1 conductance. When KCNQ1 was expressed in oocytes, an increase of external K⁺ from 0 to 10 mM K⁺ resulted in an increase of single-channel conductance from 1.9 to 4.8 pS, i.e., the resulting apparent channel conductance increased with increasing external K⁺ concentration (60). These specific characteristics of our experimental conditions may have also contributed to the increased K⁺ conductance observed.

Taking these specific features into consideration, a still obvious candidate for large-conductance K⁺ channels would be the BK channels. However, charybdotoxin, a specific inhibitor of BK channels (35), had no effect (Table 1). In contrast, 293B and HMR-1556, specific blockers of KCNQ1 channels (15, 17, 32), inhibited K⁺ currents. Strikingly, the concentrations of 293B and HMR-1556 required to inhibit K⁺-selective channels of ZG membranes were high (100–200 μM) (Figs. 2D, 2E, 4D, and 4E), which differs from the low micromolar to submicromolar concentrations required to inhibit the typical Kᵦ associated with KCNQ1 (17, 32). This is the case because KCNE1 has been shown to enhance the sensitivity of KCNQ1 to 293B and HMR-1556 (32). It could be argued that HMR-1556 and 293B are not absolutely specific blockers of KCNQ1, particularly at the high concentrations used in the present study. So far, however, only one study has been reported in which a non-KCNQ1 K⁺ channel has been shown to be blocked by both compounds in the concentration range used in our study, namely, the transient outward potassium current (Iₒ) associated with Kv4.3 (53). However, Kv4.3 is exclusively expressed in the heart and brain (42). In contrast, it is well established that KCNQ1 is expressed in pancreatic acini (12, 56). Hence, it is very likely that HMR-1556 and 293B block K⁺ currents of ZG membranes that are mediated by KCNQ1.

The specific biophysical and pharmacological properties of KCNQ1 are determined by its regulatory β-subunits KCNE1 (IsK, minK), KCNE2 (MiRP1), and KCNE3 (MiRP2), which are expressed in a tissue-specific manner to form the native K⁺ channel (36). In the heart and inner ear, KCNQ1 interacts with KCNE1 to produce a voltage-gated Kᵦ (3, 39). In contrast, coassembly of KCNQ1 with KCNE3 yields currents that are nearly instantaneous and depend linearly on voltage (40). As shown in Figs. 2C and 4C, 293B- and HMR-1556-sensitive ZG membrane K⁺ channels incorporated into planar bilayers showed a linear current-voltage relationship, suggesting that KCNQ1 expressed in ZG membranes is not associated with KCNE1. Both KCNQ1 and KCNE1 are expressed in rodent pancreatic acinar cells (12, 29, 47), but KCNQ1 may be associated with other yet unknown regulatory subunits, which could account for activation of the K⁺ current by cAMP or Ca²⁺ (4). We have previously described the association of a regulatory subunit of ZG K⁺ conductance, ZG-16p, with the ZG membrane (6). This protein is exclusively expressed in colon, pancreas, duodenum, and stomach (9). Whether ZG-16p represents a novel β-subunit of KCNQ1 in gastrointestinal epithelia remains to be investigated. These considerations are important because they could account for the different biophysical properties of the channels incorporated into the planar bilayer (e.g., K⁺ channel groups I and III shown in Figs. 2 and 4) and could reflect the association of KCNQ1 with different β-subunits or the dissociation of β-subunits after incorporation of the channels into the bilayer. The relative heterogeneity of the biophysical properties of the K⁺ channels detected could also reflect to some extent contamination from other organelles, although it is unlikely because the preparation of ZG used yield membranes of high purity (51).

Finally, we noticed the occurrence of subconduction levels in all three K⁺ channel groups (groups I-III) but particularly in group III (Figs. 2A, 3A, and 4A). These short-lived subconduction levels were mostly visited when the channel gate moved between the fully open state and the closed state. So far, there has been no report in the literature referring to a possible molecular mechanism underlying these subconduction levels for KCNQ1 K⁺ channels. As for all other six-membrane-spanning K⁺ channels, it is believed that assembly of four KCNQ1 proteins is required to form a functional channel. Interestingly, it has been suggested for another six-membrane-spanning K⁺ channel (the Kᵥ2.1) that, when channels move from the closed state to the fully open state, these sublevels result from heteromeric pore conformations, which are more frequently observed in partially activated channels, in which some but not all subunits have undergone voltage-dependent conformational changes required for channel opening (8). Hence, similar mechanisms could be operative in KCNQ1 K⁺ channels to account for subconduction levels.

**Physiological significance of ZG K⁺ channel.** There is a precedent for the expression of KCNQ1 in intracellular secretory vesicles in the gastrointestinal tract. Recently, KCNQ1 has been identified as a K⁺ channel located in intracellular tubu-
lovesicles and apical membrane of parietal cells, where it colocalizes with the H^+-K^+-ATPase (18). Inhibition of KCNQ1 current by chromanol 293B abolished acid secretion. The β-subunits KCNE2 and KCNE3 were expressed in stomach; KCNE1, however, was not. This suggested that KCNQ1 is the pore-forming subunit of the K\(^{+}\) channel responsible for sustained HCl secretion (18). Thus, in parietal cells, KCNQ1 appears to have a dual subcellular distribution by trafficking between intracellular tubulovesicles and the apical plasma membrane.

In Cl\(^{-}\) secretory epithelia, such as the colon and pancreas, a 293B-sensitive small conductance (1–2 pS) Ca\(^{2+}\)-activated K\(^{+}\) current that is located in the basolateral plasma membrane has been proposed to provide the driving force for luminal Cl\(^{-}\) secretion that may be mediated by KCNQ1 (26, 27, 29). KCNQ1 expression in the basolateral membrane of rodent pancreatic acini was subsequently confirmed by immunofluorescence microscopy using the rabbit polyclonal antibody BLE 2-1 (56) (see also Fig. 5A). It is conceivable that KCNQ1 K\(^{+}\) channels expressed in ZG membranes could also contribute to this plasma membrane K\(^{+}\) current after fusion of ZG with the apical plasma membrane. In a recent study, Lee et al. (31) tested the effect of 293B and HMR-1556 on fluid and enzyme secretion induced by ACh in the vascularity perfused rat pancreas. However, they were unable to observe any inhibitory effect of the KCNQ1 channels blockers on secretion and concluded that KCNQ1 is not essential for Ca\(^{2+}\)-mediated secretion of rat pancreatic acini. These data are at odds with their former studies on Cl\(^{-}\) secretion in isolated acini (26, 27) and could hint at additional factors that modify acinar secretion in the intact pancreas. At first sight, they also appear to contradict our present results concerning the role of KCNQ1 in enzyme secretion (Fig. 6). However, from our previous (reviewed in Ref. 48) and present studies, it appears that the membrane of ZG is equipped with at least two cation and two anion conductive pathways, which contribute to K\(^{+}\) and Cl\(^{-}\) fluxes into the granules and promote release of digestive enzymes. This apparent “functional redundancy” of ion channels in secretory granules appears to be a more widespread phenomenon. “Knockout” (/−/−) studies of CIC-3, a Cl\(^{-}\) channel, which promotes acidification of synaptic vesicles (44), show that CIC-3(−/−) synaptic vesicles still acidify, although at a lower rate, suggesting the contribution of other Cl\(^{-}\) channels to vesicular acidification. Furthermore, late endosomes and lysosomes of osteoclasts from CIC-7(−/−) mice show normal acidification rates, although the Cl\(^{-}\) channel CIC-7 is expressed in these organelles (28). Similarly, we found that CCK-stimulated enzyme secretion in permeabilized rat pancreatic acini is abolished only if the inhibitor of nonselective cation conductive pathway, flufenamate, is applied together with the KCNQ1 K\(^{+}\) channel inhibitor 293B (Fig. 6), which would reconcile our data with the results obtained by Lee et al. (31) for ACh-induced amylase secretion.

Previous studies have shown that CCK-OP- or carbachol-induced enzyme secretion of isolated rat pancreatic acini is absolutely dependent on Cl\(^{-}\): omission of Cl\(^{-}\) in the cytosol or inhibition of ZG Cl\(^{-}\) channels abolished enzyme secretion (14). Flufenamate has been shown to block CFTR Cl\(^{-}\) channels and other channels (see for instance Ref. 33), but it does not block Cl\(^{-}\)-dependent enzyme secretion as shown in Fig. 6 (compare experiments without 293B in left and right). Moreover, in the osmotic lysis assay of pancreatic ZGs, flufenamate appears to be the only compound that specifically blocks the nonselective cation permeability but not K\(^{+}\) permeability (reviewed in Ref. 48) and in addition has no effect on ZG Cl\(^{-}\) conductive pathway at concentrations up to 100 μM (data not shown). Hence, flufenamate appears to be a useful pharmacological tool in our secretion experiments, which helps to unravel the fact that both nonselective and K\(^{+}\) conductance contribute to enzyme secretion. This observation therefore hints at a functional overlap of both monovalent cation conductive pathways with regard to stimulated enzyme secretion. Thus, at the present stage of our knowledge, we must also consider that under specific (yet undetermined) physiological conditions one or the other conductance will dominate the secretory process.

To conclude, in the present study, we tested the hypothesis that KCNQ1 channels, or channels containing KCNQ1 subunits, contribute to a charge-compensating K\(^{+}\)-conducting pathway in the membrane of pancreatic ZGs. The data indicate that KCNQ1 protein is expressed in ZG membranes of the rat exocrine pancreas. KCNQ1 may account for K\(^{+}\)-selective currents, which were detected after incorporation of ZG membranes into planar bilayer membranes and blocked by the specific blockers of KCNQ1-associated K\(^{+}\) currents, 293B and HMR-1556. However, the unusual properties of the K\(^{+}\) channels at the single-channel level and the relatively low potency of the drugs tested on these channels indicate that additional subunits may modify the channel characteristics. This aspect of the study therefore requires further investigation. We propose that the K\(^{+}\) channels associated with KCNQ1 may underlie ZG K\(^{+}\)-selective monovalent K\(^{+}\) conductive pathways of ZG and contribute to secretagogue-induced enzyme secretion from pancreatic acini. Further investigations aimed at the electrophysiological and molecular characterization of additional cation and anion conductive pathways in ZGs are also required, which could lead to a better understanding of the physiology of exocrine pancreatic enzyme secretion.

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